



-PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

CAHOON ET AL.

CASE NO: BB1295 US CNT

SERIAL NO: 10/690,994

GROUP ART UNIT: 1638

FILED: OCTOBER 21, 2003

EXAMINER: L. ZHENG

FOR: PLANT DIACYLGLYCEROL  
ACYLTRANSFERASES

**DECLARATION UNDER 37 C.F.R. § 1.132**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

I, Keith R. Roesler, declare that:

I am a citizen of the United States and reside at 3928 Patricia Drive,  
Urbandale, Iowa, 50322.

I am an employee of Pioneer Hi-Bred International, Inc., ("Pioneer") a  
subsidiary of E.I. du Pont de Nemours and Company ("DuPont").

I received a Ph.D. in Agronomy from the University of Illinois at Urbana-  
Champaign. I have worked for Pioneer from July 1, 1995, to the present in the  
fields of biochemistry and molecular biology.

I am familiar with the subject matter of the above-identified application and  
supervised the performance of the experiments explained below.

The following are my remarks:

1. In the December 18, 2006, Non-Final Office Action related to the above-identified application, the Examiner *inter alia* rejected claims 26-40 under 35 U.S.C. § 101 because the claimed invention is allegedly not supported by either a specific and/or substantial asserted utility or a well-established utility.
2. Described herein are a series of experiments that establish a specific and substantial asserted utility for the claimed inventions.

3. The basic experimental procedure is as follows. A *Saccharomyces cerevisiae* strain was created with two genes deleted: the *DGA1* gene, encoding acyl-CoA:diacylglycerol acyltransferase (DGAT), and the *LRO1* gene, encoding phospholipid:diacylglycerol acyltransferase (PDAT). In this yeast strain, DGAT genes were overexpressed using a strong constitutive promoter from the yeast phosphoglycerate kinase gene, and using uracil selection. DGAT assays were done using microsomal membrane preps.
4. The method of Milcamps *et al.*, 2005, "Isolation of a gene encoding a 1,2-diacylglycerol-*sn*-acetyl-CoA acetyltransferase from developing seeds of *Euonymus alatus*", J. Biol. Chem. 280:5370-5377 (attached herewith), was followed, with minor changes. *Saccharomyces cerevisiae* cultures were grown to early stationary phase in 100 ml of SC media minus uracil. Following harvest, the yeast pellets were resuspended in 4 ml of 20 mM Tris-HCl, pH 8, 10 mM MgCl<sub>2</sub>, 1 mM EDTA, 5% glycerol, 1 mM DTT, and 0.3 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. Two ml of glass beads were added, and cells were lysed by vortexing for 5 min. The lysate was centrifuged for 15 min at 1500 g at 6 °C. The supernatant was then centrifuged at 100,000 g for 1.5 hr at 6 °C. The microsomal pellet was resuspended in 500 µl of 100 mM potassium phosphate, pH 7.2, containing 10% glycerol and frozen in liquid nitrogen prior to storage at -80 °C. Protein concentrations were determined by the method of Bradford, using the Coomassie Plus reagent (Pierce), with bovine serum albumin as standard.
5. DGAT assays were done for 1 min at 25 °C with 50 mM potassium phosphate pH 7.2, 10 µM 1-<sup>14</sup>C-labeled oleoyl-coenzyme A (50 mCi/mmol, Perkin Elmer), and 20 µg of microsomal protein, using endogenous diacylglycerol, in a total reaction volume of 100 µl. The reaction was started by addition of the microsomal membranes to the remainder of the reaction components. The assay was stopped and lipids were extracted with 2 ml of hexane:isopropanol (3:2) (Hara and Radin, 1978, "Lipid extraction of tissues with a low-toxicity solvent", Anal. Biochem. 90:420-426) containing 4 µl of unlabeled triacylglycerol (triolein, Sigma). Following vortexing for 10 sec, the phases were separated with 1 ml of 500 mM sodium sulfate and vortexing was again done for 10 sec. After 10 min, the upper phase was transferred to another tube and dried with nitrogen gas. The lipid was resolubilized in a small volume of hexane (approximately 100 to

150 µl) and applied to K6 silica TLC plates, which were developed in 80:20:1 (v/v/v) hexane:diethylether:acetic acid. Triacylglycerol was visualized and marked by staining in iodine vapor. After the stain faded, the triacylglycerol was scraped, and radioactivity was determined by liquid scintillation counting. The following results were obtained.

Construct	DGAT Activity (pmol <sup>14</sup> C-labeled TAG produced/min/mg microsomal protein)			
	Rep1	Rep2	Rep3	Mean ± SD
Soybean DGAT	840	887	876	868 ± 25
Wheat DGAT	518	529	515	521 ± 7
Vector control	33	14	17	21 ± 10

6. The soybean DGAT protein-coding region was obtained by PCR using clone sr1.pk0098.a8 as a template. This sequence was used to create the yeast expression vector, PHP32069 (Appendix A). The sequence of the soybean DGAT-coding region, "PHP32069 Soy DGAT1 CDS", was found to have a one nucleotide difference with the corresponding DGAT-coding region of SEQ ID NO:15, "BB1295 SEQ-15 CDS". An alignment of the two DGAT-coding sequences is presented in Appendices B1-B4. The soybean DGAT sequence in PHP32069 has an adenine (A) at position 939 of the DGAT-coding region, while the corresponding nucleotide in SEQ ID NO:15 is cytosine (C). The cDNA insert in clone sr1.pk0098.a8 was re-sequenced, and the sequence of the DGAT-coding region in sr1.pk0098.a8 was identical to the sequence of the PCR-derived DGAT-coding region in PHP32069. The single nucleotide difference in SEQ ID NO:15, also obtained by sequencing clone sr1.pk0098.a8, is presumably due to a sequencing error. This single nucleotide difference results in a one amino acid difference between the two corresponding amino acid sequences (Appendix C). The DGAT protein encoded by PHP32069 has a glutamic acid residue (E) at position 313, while the corresponding residue in SEQ ID NO:16 is aspartic acid (D).

7. The wheat DGAT DNA was obtained by a combination of PCR using clone wr1.pk0119.b6: fis as a template and a synthetic gene fragment to complete the coding region. This DGAT-coding sequence was used to create the yeast expression vector, PHP32068 (Appendix D). The sequence of this wheat DGAT-

coding region, "PHP32068 Wheat DGAT1-2 CDS", was found to have two single nucleotide differences with the corresponding DGAT-coding region of SEQ ID NO:21, "BB1295 SEQ-21 CDS". An alignment of the two DGAT-coding sequences is presented in Appendices E1-E4. The wheat DGAT nucleotide sequence of PHP32068 has a guanosine (G) at position 303 and a thymidine (T) at position 393; the sequence of SEQ ID NO:21 has a thymidine (T) and cytosine (C) at these two positions, respectively. These two nucleotide differences are "silent", i.e., the amino acid sequence encoded by PHP32068 is identical to that of SEQ ID NO:22 (Appendix F).

8. Sequence alignments were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal V method of alignment with the default parameters

9. The vector control contained no DGAT gene.

10. As shown in the table above, both the soybean DGAT protein encoded by PHP32069 and the wheat DGAT protein encoded by PHP32068 have significant DGAT activity.

11. I believe that the experiments conducted thus establish a specific and substantial utility for the claimed inventions.

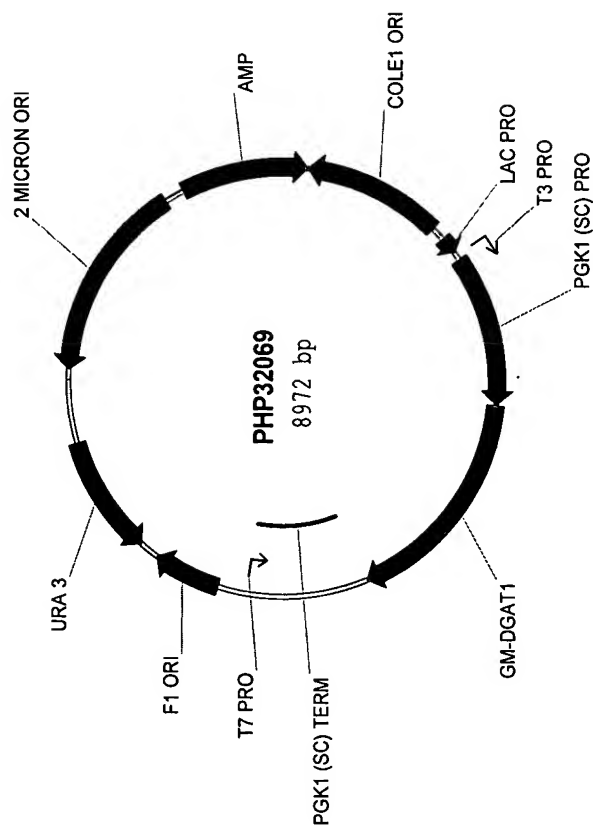
12. I declare that all statements made herein are either based on my own knowledge and are true, or if based on information and belief are believed to be true. I also declare that all statements were made with knowledge that willful false statements, and the like, are punishable by either fine, or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and any such willful false statements may jeopardize the validity of either the patent application, or any patent issuing thereon.

By: Keith R. Roesler  
Keith R. Roesler

Dated: 5-14-07



# APPENDIX A YEAST EXPRESSION VECTOR FOR SOYBEAN DGAT



APPENDIX B1

SOYBEAN DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

	A	T	G	G	C	G	A	T	T	T	C	C	G	A	T	G	A	A	A	G	T	G	T	A	G	C	C	A	C	T	G	C	T	C	T	C	A	A	C	A	C	T	C		Consensus #1				
						10										20																														50			
1	A	T	G	G	C	G	A	T	T	T	C	C	G	A	T	G	A	A	A	G	T	G	T	A	G	C	C	A	C	T	G	C	T	C	T	C	A	A	C	A	C	T	C		BB1295 SEQ-15 CDS.seq				
1	A	T	G	G	C	G	A	T	T	T	C	C	G	A	T	G	A	A	A	G	T	G	T	A	G	C	C	A	C	T	G	C	T	C	T	C	A	A	C	A	C	T	C		PHP32069 Soy DGAT1 CDS.seq				
	T	T	C	C	T	G	C	G	C	C	G	T	C	C	T	C	C	G	C	A	C	T	C	C	A	C	C	G	C	C	T	C	T	C	T	C	T	C	A	A	T	T		Consensus #1					
						60										70																														100			
51	T	T	C	C	T	G	C	G	C	C	G	T	C	C	C	T	C	G	C	C	A	C	T	C	C	C	C	C	C	C	T	C	T	C	T	C	T	C	A	A	T	T		BB1295 SEQ-15 CDS.seq					
51	T	T	C	C	T	G	C	G	C	C	G	T	C	C	C	T	C	G	C	C	A	C	T	C	C	C	C	C	C	C	T	C	T	C	T	C	A	A	T	T		PHP32069 Soy DGAT1 CDS.seq							
	C	G	C	T	G	A	G	A	C	A	C	C	A	C	G	A	C	C	A	G	T	T	C	C	G	T	G	A	T	G	A	C	T	T	G	G	C	A	A	G	A	T	T		Consensus #1				
						110										120																														150			
101	C	G	C	T	G	A	G	A	C	A	C	C	A	C	C	A	C	A	C	A	G	T	T	C	C	G	T	G	A	T	G	A	C	T	T	G	G	C	C	A	A	G	A	T	T		BB1295 SEQ-15 CDS.seq		
101	C	G	C	T	G	A	G	A	C	A	C	C	A	C	C	A	C	A	C	A	G	T	T	C	C	G	T	G	A	T	G	A	C	T	T	G	G	C	C	A	A	G	A	T	T		PHP32069 Soy DGAT1 CDS.seq		
	G	G	T	T	C	C	G	A	C	T	C	C	A	T	C	A	A	C	A	G	C	G	A	C	G	C	C	G	T	C	A	A	T	T	C	C	A	A	T	T	C	C	A	A	C	A		Consensus #1	
						160										170																														200			
151	G	G	T	T	C	C	G	A	C	T	C	C	A	T	C	A	A	C	A	G	C	G	A	C	G	C	C	G	C	C	G	T	C	A	A	T	T	C	C	A	A	C	A	C	A		BB1295 SEQ-15 CDS.seq		
151	G	G	T	T	C	C	G	A	C	T	C	C	A	T	C	A	A	C	A	G	C	G	A	C	G	C	C	G	C	C	G	T	C	A	A	T	T	C	C	A	A	C	A	C	A		PHP32069 Soy DGAT1 CDS.seq		
	G	C	A	A	C	G	A	A	A	C	A	A	C	A	C	T	G	A	T	T	T	C	T	C	C	G	T	C	C	A	A	T	T	C	G	C	T	A	A	T	T	C	G	C	T	A	C		Consensus #1
						210										220																														250			
201	G	C	A	A	C	G	A	A	A	C	A	A	C	A	C	A	C	A	A	G	T	T	T	C	C	G	T	C	C	T	C	A	A	T	T	C	G	C	C	T	A	C		BB1295 SEQ-15 CDS.seq					
201	G	C	A	A	C	G	A	A	A	C	A	A	C	A	C	A	C	A	A	G	T	T	T	C	C	G	T	C	C	T	C	A	A	T	T	C	G	C	C	T	A	C		PHP32069 Soy DGAT1 CDS.seq					
	G	T	C	C	T	T	C	C	C	G	T	C	A	C	C	G	C	A	A	G	T	G	A	A	G	T	C	C	G	T	C	A	A	T	T	C	G	C	T	C	A	G	T	C		Consensus #1			
						260										270																														300			
251	G	T	C	C	T	T	C	C	G	T	C	C	C	T	C	A	C	C	G	C	A	A	G	T	G	A	A	G	T	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C		BB1295 SEQ-15 CDS.seq			
251	G	T	C	C	T	T	C	C	G	T	C	C	C	T	C	A	C	C	G	C	A	A	G	T	G	A	A	G	T	C	C	C	C	C	C	C	C	C	C	C	C	C	C		PHP32069 Soy DGAT1 CDS.seq				
	G	A	C	A	C	T	A	T	T	T	C	C	G	T	C	A	G	A	G	T	C	A	C	G	G	C	C	T	C	T	T	C	A	A	C	T	T	G	T	A	T	A	G	T		Consensus #1			
						310										320																														350			
301	G	A	C	A	C	T	A	T	T	T	C	C	G	T	C	A	G	A	G	T	C	A	C	G	C	G	C	C	T	C	T	T	C	A	A	C	T	T	G	T	A	T	A	G	T		BB1295 SEQ-15 CDS.seq		
301	G	A	C	A	C	T	A	T	T	T	C	C	G	T	C	A	G	A	G	T	C	A	C	G	C	G	C	C	T	C	T	T	C	A	A	C	T	T	G	T	A	T	A	G	T		PHP32069 Soy DGAT1 CDS.seq		
	A	G	T	C	C	T	T	G	T	G	T	G	T	G	A	T	A	G	C	C	A	C	T	C	A	T	T	G	A	G	A	T	T	A	T	G	A	A	T	T	A	T	G	A	A	G	T		Consensus #1
						360										370																														400			
351	A	G	T	C	C	T	T	G	T	G	T	G	T	G	A	T	A	G	C	C	G	A	C	T	C	A	T	T	G	A	G	A	T	T	A	T	T	A	T	T	A	T	G	A	A	G	T		BB1295 SEQ-15 CDS.seq
351	A	G	T	C	C	T	T	G	T	G	T	G	T	G	A	T	A	G	C	C	G	A	C	T	C	A	T	T	G	A	G	A	T	T	A	T	T	A	T	T	A	T	G	A	A	G	T		PHP32069 Soy DGAT1 CDS.seq

# APPENDIX B2

[illegible]

# APPENDIX B3

# SOYBEAN DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

[illegible]



# APPENDIX B4

# SOYBEAN DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

	A	T	C	C	C	C	A	T	A	T	T	T	C	C	A	T	T	T	A	A	G	C	A	C	A	A	A	G	G	C	T	G	C	Consensus #1				
	1210	1220	1230	1240	1250																																	
1201	A	T	C	C	C	C	A	C	T	A	T	T	T	C	C	A	T	G	T	T	A	G	C	A	C	C	A	A	G	C	T	G	C	BA1295 SEQ-15 CDS.seq				
1201	A	T	C	C	C	C	A	T	A	T	T	T	T	A	A	G	C	A	C	G	T	C	T	A	C	C	A	A	G	C	T	G	C	PHP32069 Soy DGAT1 CDS.seq				
	1260	1270	1280	1290	1300																																	
1251	T	G	C	T	T	T	A	T	G	C	C	T	T	C	T	T	C	T	T	A	T	T	C	C	A	T	G	A	G	C	T	G	C	Consensus #1				
1251	T	G	C	T	T	T	A	T	G	C	C	T	T	C	T	T	C	T	T	A	T	T	C	C	A	T	G	A	G	C	T	G	C	BA1295 SEQ-15 CDS.seq				
1251	T	G	C	T	T	T	A	T	G	C	C	T	T	C	T	T	C	T	T	A	T	T	C	C	A	T	G	A	G	C	T	G	C	PHP32069 Soy DGAT1 CDS.seq				
	1310	1320	1330	1340	1350																																	
1301	T	T	C	A	G	G	T	T	C	C	T	T	T	G	G	T	C	A	C	T	A	T	T	T	C	G	G	T	G	A	A	T	T	A	Consensus #1			
1301	T	T	C	A	G	G	T	T	C	C	T	T	T	G	G	T	T	C	A	A	G	T	T	T	C	G	G	T	T	G	A	A	T	T	BA1295 SEQ-15 CDS.seq			
1301	T	T	C	A	G	G	T	T	C	C	T	T	T	G	G	T	T	C	A	A	G	T	T	T	C	G	G	T	T	G	A	A	T	T	PHP32069 Soy DGAT1 CDS.seq			
	1360	1370	1380	1390	1400																																	
1351	T	T	C	A	G	G	T	T	C	C	T	T	T	G	G	T	C	A	C	T	A	T	T	T	C	G	C	A	A	A	T	T	T	C	A	Consensus #1		
1351	T	T	C	A	G	G	T	T	C	C	T	T	T	G	G	T	T	C	A	C	T	A	T	T	T	C	G	C	A	A	A	T	T	T	C	BA1295 SEQ-15 CDS.seq		
1351	T	T	C	A	G	G	T	T	C	C	T	T	T	G	G	T	T	C	A	C	T	A	T	T	T	C	G	C	A	A	A	T	T	T	C	PHP32069 Soy DGAT1 CDS.seq		
	1410	1420	1430	1440	1450																																	
1401	A	A	C	T	C	A	A	T	G	G	T	T	T	T	G	G	T	T	C	A	T	T	T	T	C	A	G	T	A	T	C	C	T	T	Consensus #1			
1401	A	A	C	T	C	A	A	T	G	G	T	T	T	T	G	G	T	T	C	A	T	T	T	T	C	A	G	T	A	T	C	C	T	T	BA1295 SEQ-15 CDS.seq			
1401	A	A	C	T	C	A	A	T	G	G	T	T	T	T	G	G	T	T	C	A	T	T	T	T	C	A	G	T	A	T	C	C	T	T	PHP32069 Soy DGAT1 CDS.seq			
	1460	1470	1480	1490	1500																																	
1451	G	T	C	A	C	C	T	A	T	G	T	G	T	A	C	T	G	C	T	A	T	A	C	T	A	C	C	A	T	G	A	T	A	G	G	A	Consensus #1	
1451	G	T	C	A	C	C	T	A	T	G	T	G	T	A	C	T	G	C	T	A	T	A	C	T	A	C	C	A	T	G	A	T	A	G	G	A	BA1295 SEQ-15 CDS.seq	
1451	G	T	C	A	C	C	T	A	T	G	T	G	T	A	C	T	G	C	T	A	T	A	C	T	A	C	C	A	T	G	A	T	A	G	G	A	PHP32069 Soy DGAT1 CDS.seq	
	G	G	C	A	A	A	C	T	G	A	C																											Consensus #1
	G	G	C	A	A	A	C	T	G	A	C																											BA1295 SEQ-15 CDS.seq
1501	G	G	C	A	A	A	C	T	G	A	C																											PHP32069 Soy DGAT1 CDS.seq

Consensus 'Consensus #1': When all match the residue of BB1295 SEQ-15 CDS.seq show the residue of BB1295 SEQ-15 CDS.seq, otherwise show '.'.

Decoration 'Decoration #1': Box residues that differ from BB1295 SEQ-15 CDS.seq.

# APPENDIX C

# SOYBEAN DGAT AMINO ACID SEQUENCE ALIGNMENT

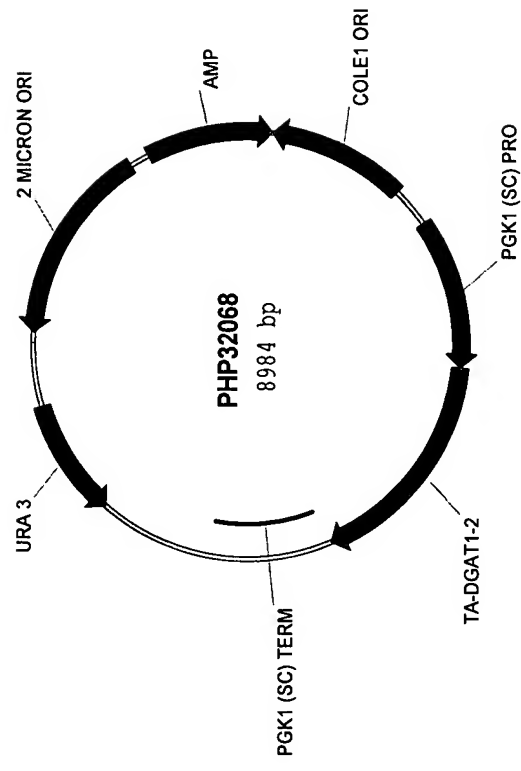
	M A I S D E P E S V A T A L N H S S L R R R P S A T S T A G L F N S P E T T I D S S C D D L A K D S G S D S I N S D D	Consensus #1
	10            20            30            40            50            60	
1	M A I S D E P E S V A T A L N H S S L R R R P S A T S T A G L F N S P E T T I D S S C D D L A K D S G S D S I N S D D	Bt1295 USCUT SEQ-16.pro
1	M A I S D E P E S V A T A L N H S S L R R R P S A T S T A G L F N S P E T T I D S S C D D L A K D S G S D S I N S D D	PHP32069 Soy DGAT1.pro
	A A V N S Q Q O N E K Q D T D F S V L K F A Y R P S V P A H R K V K E S P L S S D T I F R O S H A G L F N L C I V V L V	Consensus #1
	70            80            90            100            110            120	
61	A A V N S Q Q O N E K Q D T D F S V L K F A Y R P S V P A H R K V K E S P L S S D T I F R Q S H A G L F N L C I V V L V	Bt1295 USCUT SEQ-16.pro
61	A A V N S Q Q O N E K Q D T D F S V L K F A Y R P S V P A H R K V K E S P L S S D T I F R Q S H A G L F N L C I V V L V	PHP32069 Soy DGAT1.pro
	A V N S R L I I E N L M K Y G W L I K S G F W F S S K S L R D W P L F M C C L S L V V P F P A A F I V E K L A Q R K C I	Consensus #1
	130            140            150            160            170            180	
121	A V N S R L I I E N L M K Y G W L I K S G F W F S S K S L R D W P L F M C C L S L V V P F P A A F I V E K L A Q R K C I	Bt1295 USCUT SEQ-16.pro
121	A V N S R L I I E N L M K Y G W L I K S G F W F S S K S L R D W P L F M C C L S L V V P F P A A F I V E K L A Q R K C I	PHP32069 Soy DGAT1.pro
	P E P V V V V L H I I I T S T S L F Y P V L V I L R C D S A F V S G V T L M L F S C V V W L K L V S Y A H T N Y D M R A	Consensus #1
	190            200            210            220            230            240	
181	P E P V V V V L H I I I T S T S L F Y P V L V I L R C D S A F V S G V T L M L F S C V V W L K L V S Y A H T N Y D M R A	Bt1295 USCUT SEQ-16.pro
181	P E P V V V V L H I I I T S T S L F Y P V L V I L R C D S A F V S G V T L M L F S C V V W L K L V S Y A H T N Y D M R A	PHP32069 Soy DGAT1.pro
	L T K L V E K G E A L L D T L N M D Y P Y N V S F K S L A Y F L V A P T L C Y Q P S Y P R T P Y I I R K G W L F R Q L V K	Consensus #1
	250            260            270            280            290            300	
241	L T K L V E K G E A L L D T L N M D Y P Y N V S F K S L A Y F L V A P T L C Y Q P S Y P R T P Y I I R K G W L F R Q L V K	Bt1295 USCUT SEQ-16.pro
241	L T K L V E K G E A L L D T L N M D Y P Y N V S F K S L A Y F L V A P T L C Y Q P S Y P R T P Y I I R K G W L F R Q L V K	PHP32069 Soy DGAT1.pro
	L I I F T G V M G F I I - Q Y I N P I V Q N S Q H P L K G N L L Y A T E R V L K L S V P N L Y V W L C M F Y C F F H L W	Consensus #1
	310            320            330            340            350            360	
301	L I I F T G V M G F I I D Q Y I N P I V Q N S Q H P L K G N L L Y A T E R V L K L S V P N L Y V W L C M F Y C F F H L W	Bt1295 USCUT SEQ-16.pro
301	L I I F T G V M G F I I <span style="border: 1px solid black;">E</span> Q Y I N P I V Q N S Q H P L K G N L L Y A T E R V L K L S V P N L Y V W L C M F Y C F F H L W	PHP32069 Soy DGAT1.pro
	L N I A E L L R F G D R E F Y K D W W N A K T V E D Y W R M W N M P V H K W M I R H L Y F P C L R H G L P K A A L L	Consensus #1
	370            380            390            400            410            420	
361	L N I A E L L R F G D R E F Y K D W W N A K T V E D Y W R M W N M P V H K W M I R H L Y F P C L R H G L P K A A L L	Bt1295 USCUT SEQ-16.pro
361	L N I A E L L R F G D R E F Y K D W W N A K T V E D Y W R M W N M P V H K W M I R H L Y F P C L R H G L P K A A L L	PHP32069 Soy DGAT1.pro
	I A F L V S A L F H E L C I A V P C H I F K L W A F G G I M F Q V P L V L I T N Y L Q N K F R N S M V G N M I F W F I F	Consensus #1
	430            440            450            460            470            480	
421	I A F L V S A L F H E L C I A V P C H I F K L W A F G G I M F Q V P L V L I T N Y L Q N K F R N S M V G N M I F W F I F	Bt1295 USCUT SEQ-16.pro
421	I A F L V S A L F H E L C I A V P C H I F K L W A F G G I M F Q V P L V L I T N Y L Q N K F R N S M V G N M I F W F I F	PHP32069 Soy DGAT1.pro
.	S I L G Q P M C V L L Y Y H D L M N R K G K L D	Consensus #1
	490            500	
481	S I L G Q P M C V L L Y Y H D L M N R K G K L D	Bt1295 USCUT SEQ-16.pro
481	S I L G Q P M C V L L Y Y H D L M N R K G K L D	PHP32069 Soy DGAT1.pro

Consensus 'Consensus #1': When all match the residue of BB1295 USCNT SEQ-16.pro show the residue of BB1295 USCNT SEQ-16.pro, otherwise show ' '.

Decoration 'Decoration #1': Box residues that differ from BB1295 USCNT SEQ-16.pro.

# APPENDIX D

## YEAST EXPRESSION VECTOR FOR WHEAT DGAT



APPENDIX E1  
WHEAT DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

	A	T	G	T	C	A	A	A	G	G	A	A	C	C	C	A	G	A	C	C	C	G	C	A	C	C	T	C	C	C	G	G	C	A	G	C	T	T	C	C	T	T	C		Consensus #1						
	10																20																													50					
1	A	T	G	T	C	A	A	A	G	G	A	A	C	C	C	A	G	A	C	C	C	G	C	A	C	C	T	T	C	C	C	G	G	C	A	G	C	T	T	C	C	T	T	C		BB1295 SEQ-21 CDS.seq					
1	A	T	G	T	C	A	A	A	G	G	A	A	C	C	C	A	G	A	C	C	C	G	C	A	C	C	T	T	C	C	C	G	G	C	A	G	C	T	T	C	C	T	T	C		PHP32068 Wheat DGAT1-2 CDS.seq					
	C	A	C	G	G	C	G	C	C	A	C	C	A	A	C	C	A	A	C	C	C	C	C	C	C	A	A	C	C	C	C	C	C	C	C	A	A	C	C	T	T	C	C	G	A		Consensus #1				
	60																70																													100					
51	C	A	C	G	G	C	G	C	C	A	C	C	A	A	C	C	A	A	C	C	C	C	C	C	A	A	C	C	C	C	C	C	C	C	C	A	A	C	C	T	T	C	C	G	A		BB1295 SEQ-21 CDS.seq				
51	C	A	C	G	G	C	G	C	C	A	C	C	A	A	C	C	A	A	C	C	C	C	C	C	A	A	C	C	C	C	C	C	C	C	C	A	A	C	C	T	T	C	C	G	A		PHP32068 Wheat DGAT1-2 CDS.seq				
	A	C	T	C	C	C	T	C	A	G	T	T	C	C	A	T	G	G	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	T	G	C	C	G	C	T	G	C		Consensus #1				
	110																120																													150					
101	A	C	T	C	C	C	T	C	A	G	T	T	C	C	A	C	C	A	A	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	A	C	C	T	T	C	C	G	C		BB1295 SEQ-21 CDS.seq			
101	A	C	T	C	C	C	T	C	A	G	T	T	C	C	A	C	C	A	A	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	A	C	C	T	T	C	C	G	C		PHP32068 Wheat DGAT1-2 CDS.seq			
	A	C	G	A	T	C	G	C	A	C	G	A	C	C	C	T	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	T	G	C	C	G	C	A	C		Consensus #1				
	160																170																													200					
151	A	C	G	A	T	C	G	C	A	C	G	A	C	C	C	C	T	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	A	C	C	T	G	C	C	G	A	C		BB1295 SEQ-21 CDS.seq		
151	A	C	G	A	T	C	G	C	A	C	G	A	C	C	C	C	T	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	A	C	C	T	G	C	C	G	A	C		PHP32068 Wheat DGAT1-2 CDS.seq		
	G	G	T	C	C	A	C	G	A	G	G	C	G	C	C	A	T	G	G	A	G	C	A	G	C	C	C	C	C	C	C	C	C	C	C	C	A	C	G	A	C	G	A	T	G		Consensus #1				
	210																220																													250					
201	G	G	T	C	C	A	C	G	A	G	G	C	G	C	C	A	T	G	G	A	G	C	A	G	C	C	C	C	C	C	C	C	C	C	C	A	C	G	A	C	G	A	T	G		BB1295 SEQ-21 CDS.seq					
201	G	G	T	C	C	A	C	G	A	G	G	C	G	C	C	A	T	G	G	A	G	C	A	G	C	C	C	C	C	C	C	C	C	C	C	A	C	G	A	C	G	A	T	G		PHP32068 Wheat DGAT1-2 CDS.seq					
	C	C	T	G	C	T	A	C	C	G	G	C	G	C	C	C	A	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	A	G	A	G	A	G	C	C	G		Consensus #1			
	260																270																													300					
251	C	C	T	G	C	T	A	C	C	G	G	C	G	C	C	C	G	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	A	G	A	G	A	G	C	C	G		BB1295 SEQ-21 CDS.seq			
251	C	C	T	G	C	T	A	C	C	G	G	C	G	C	C	C	G	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	A	G	A	G	A	G	C	C	G		PHP32068 Wheat DGAT1-2 CDS.seq			
	C	T	.	A	G	C	T	C	C	G	A	C	G	C	C	A	T	C	C	G	A	C	A	G	C	C	C	C	C	C	C	C	C	C	C	C	A	T	G	C	A	G	A	T	C		Consensus #1				
	310																320																													350					
301	C	T	.	A	G	C	T	C	C	G	A	C	G	C	C	A	T	C	C	G	A	C	A	G	C	C	C	C	C	C	C	C	C	C	C	C	A	T	G	C	A	G	A	T	C		BB1295 SEQ-21 CDS.seq				
301	C	T	.	A	G	C	T	C	C	G	A	C	G	C	C	A	T	C	C	G	A	C	A	G	C	C	C	C	C	C	C	C	C	C	C	C	A	T	G	C	A	G	A	T	C		PHP32068 Wheat DGAT1-2 CDS.seq				
	A	T	G	C	A	T	T	G	T	G	C	T	G	A	T	T	G	C	A	G	T	G	A	C	A	G	C	C	C	C	C	C	C	C	C	C	A	T	T	A	T	.	G	A	G	A	A	C	T		Consensus #1
	360																370																													400					
351	A	T	G	C	A	T	T	G	T	G	C	T	G	A	T	T	G	C	A	G	T	G	A	C	A	G	C	C	C	C	C	C	C	C	C	A	T	T	A	T	C	G	A	G	A	A	C	T		BB1295 SEQ-21 CDS.seq	
351	A	T	G	C	A	T	T	G	T	G	C	T	G	A	T	T	G	C	A	G	T	G	A	C	A	G	C	C	C	C	C	C	C	C	C	A	T	T	A	T	C	G	A	G	A	A	C	T		PHP32068 Wheat DGAT1-2 CDS.seq	

APPENDIX E2

WHEAT DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

	T	A	T	G	A	G	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A
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# APPENDIX E3

[illegible]

APPENDIX E4  
WHEAT DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

	A A G T G G A T C G T T C G A C A T A T A T A T T T T C C A T G C A T A A G G A A T G G C T T A T T C	1210	1220	1230	1240	Consensus #1
1201	A A G T G G A T C G T T C G A C A T A T A T A T T T T C C A T G C A T A A G G A A T G G C T T A T T C					BB1295 SEQ-21 CDS.seq
1201	A A G T G G A T C G T T C G A C A T A T A T A T T T T C C A T G C A T A A G G A A T G G C T T A T T C					PHF32068 Wheat DGAT1-2 CDS.seq
	A A A G G G T G T G C C A T T C T C A T C G C C A T T T C T G G T T T C A G C T G T A T T T C A T G	1250	1260	1270	1280	Consensus #1
1251	A A A G G G T G T G C C A T T C T C A T C G C C A T T T C T G G T T T C A G C T G T A T T T C A T G					BB1295 SEQ-21 CDS.seq
1251	A A A G G G T G T G C C A T T C T C A T C G C C A T T T C T G G T T T C A G C T G T A T T T C A T G					PHF32068 Wheat DGAT1-2 CDS.seq
	A G C T A T G T A T T G C T G T T C C G T G C C A C A T T T T C A A A T T A T G G G C A T T T T C T	1310	1320	1330	1340	Consensus #1
1301	A G C T A T G T A T T G C T G T T C C G T G C C A C A T T T T C A A A T T A T G G G C A T T T T C T					BB1295 SEQ-21 CDS.seq
1301	A G C T A T G T A T T G C T G T T C C G T G C C A C A T T T T C A A A T T A T G G G C A T T T T C T					PHF32068 Wheat DGAT1-2 CDS.seq
	G G A A T C A T G T T T C A G A T T C C C C T G C T A T T C T T G A C G A A A T A T C T T C A A G A	1350	1360	1370	1380	Consensus #1
1351	G G A A T C A T G T T T C A G A T T C C C C T G C T A T T C T T G A C G A A A T A T C T T C A A G A					BB1295 SEQ-21 CDS.seq
1351	G G A A T C A T G T T T C A G A T T C C C C T G C T A T T C T T G A C G A A A T A T C T T C A A G A					PHF32068 Wheat DGAT1-2 CDS.seq
	T A A G T T C A A G A A T A C A A T G G T G G G C A C A T G A T A T T T G G T T C T T C T C A	1410	1420	1430	1440	Consensus #1
1401	T A A G T T C A A G A A T A C A A T G G T G G G C A C A T G A T A T T T G G T T C T T C T C A					BB1295 SEQ-21 CDS.seq
1401	T A A G T T C A A G A A T A C A A T G G T G G G C A C A T G A T A T T T G G T T C T T C T C A					PHF32068 Wheat DGAT1-2 CDS.seq
	G C A T A G T G G G C A C C A A T G T G T G T T C T C T T G T A C T A C C A T G A T G T C A T G	1450	1460	1470	1480	Consensus #1
1451	G C A T A G T G G G C A C C A A T G T G T G T T C T C T T G T A C T A C C A T G A T G T C A T G					BB1295 SEQ-21 CDS.seq
1451	G C A T A G T G G G C A C C A A T G T G T G T T C T C T T G T A C T A C C A T G A T G T C A T G					PHF32068 Wheat DGAT1-2 CDS.seq
	A C A G A C A G G C T C A G A C A A T G G C	1510	1520			Consensus #1
1501	A C A G A C A G G C T C A G A C A A T G G C					BB1295 SEQ-21 CDS.seq
1501	A C A G A C A G G C T C A G A C A A T G G C					PHF32068 Wheat DGAT1-2 CDS.seq

Consensus 'Consensus #1': When all match the residue of BB1295 SEQ-21 CDS.seq show the residue of BB1295 SEQ-21 CDS.seq, otherwise show '','.

Decoration 'Decoration #1': Box residues that differ from BB1295 SEQ-21 CDS.seq.

# APPENDIX F – WHEAT DGAT AMINO ACID SEQUENCE ALIGNMENT

	M	S	K	G	N	P	D	P	H	L	P	G	S	F	L	P	S	H	G	G	P	P	P	K	P	K	T	P	P	R	T	F	R	N	L	P	S	S	S	T	H	G	P	A	P	S	V	A	A	A		Consensus #1
	10																																																			
	10																																																			
1	M	S	K	G	N	P	D	P	H	L	P	G	S	F	L	P	S	H	G	G	P	P	P	K	P	K	T	P	P	R	T	F	R	N	L	P	S	S	S	T	H	G	P	A	P	S	V	A	A	A		BB1295 USCNT SEQ-22.pro
1	M	S	K	G	N	P	D	P	H	L	P	G	S	F	L	P	S	H	G	G	P	P	P	K	P	K	T	P	P	R	T	F	R	N	L	P	S	S	S	T	H	G	P	A	P	S	V	A	A	A		PHF32068 Wheat DGAT1-2.pro
	60																																																			
	60																																																			
	T	I	A	T	T	P	S	A	A	P	L	P	T	V	H	G	E	A	A	H	G	A	A	A	A	A	A	A	A	R	R	D	A	L	L	P	G	V	G	A	A	H	R	R	V	K	E	S	P		Consensus #1	
	100																																																			
	100																																																			
51	T	I	A	T	T	P	S	A	A	P	L	P	T	V	H	G	E	A	A	H	G	A	A	A	A	A	A	A	R	R	D	A	L	L	P	G	V	G	A	A	H	R	R	V	K	E	S	P		BB1295 USCNT SEQ-22.pro		
51	T	I	A	T	T	P	S	A	A	P	L	P	T	V	H	G	E	A	A	H	G	A	A	A	A	A	A	A	R	R	D	A	L	L	P	G	V	G	A	A	H	R	R	V	K	E	S	P		PHF32068 Wheat DGAT1-2.pro		
	130																																																			
	130																																																			
	L	S	S	D	A	I	F	R	Q	S	H	A	G	L	N	L	C	I	V	V	L	I	A	V	N	S	R	L	I	E	N	L	M	K	Y	G	L	L	I	R	A	G	F	W	F	E	S	A	R		Consensus #1	
	150																																																			
	150																																																			
101	L	S	S	D	A	I	F	R	Q	S	H	A	G	L	N	L	C	I	V	V	L	I	A	V	N	S	R	L	I	E	N	L	M	K	Y	G	L	L	I	R	A	G	F	W	F	E	S	A	R		BB1295 USCNT SEQ-22.pro	
101	L	S	S	D	A	I	F	R	Q	S	H	A	G	L	N	L	C	I	V	V	L	I	A	V	N	S	R	L	I	E	N	L	M	K	Y	G	L	L	I	R	A	G	F	W	F	E	S	A	R		PHF32068 Wheat DGAT1-2.pro	
	180																																																			
	180																																																			
	S	L	G	D	W	P	L	L	M	C	C	L	T	L	P	I	F	P	L	A	A	L	M	T	E	K	W	A	Q	R	K	L	I	R	D	H	V	S	I	L	L	H	I	I	T	T	V	L		Consensus #1		
	200																																																			
	200																																																			
151	S	L	G	D	W	P	L	L	M	C	C	L	T	L	P	I	F	P	L	A	A	L	M	T	E	K	W	A	Q	R	K	L	I	R	D	H	V	S	I	L	L	H	I	I	T	T	V	L		BB1295 USCNT SEQ-22.pro		
151	S	L	G	D	W	P	L	L	M	C	C	L	T	L	P	I	F	P	L	A	A	L	M	T	E	K	W	A	Q	R	K	L	I	R	D	H	V	S	I	L	L	H	I	I	T	T	V	L		PHF32068 Wheat DGAT1-2.pro		
	230																																																			
	230																																																			
	I	Y	P	V	V	I	L	K	C	E	S	A	V	L	S	G	F	V	L	M	F	I	A	S	I	T	W	L	K	L	V	S	F	A	H	T	N	Y	D	I	R	L	S	Q	S	I	E	K		Consensus #1		
	250																																																			
	250																																																			
201	T	Y	P	V	V	I	L	K	C	E	S	A	V	L	S	G	F	V	L	M	F	I	A	S	I	T	W	L	K	L	V	S	F	A	H	T	N	Y	D	I	R	L	S	Q	S	I	E	K		BB1295 USCNT SEQ-22.pro		
201	I	Y	P	V	V	I	L	K	C	E	S	A	V	L	S	G	F	V	L	M	F	I	A	S	I	T	W	L	K	L	V	S	F	A	H	T	N	Y	D	I	R	L	S	Q	S	I	E	K		PHF32068 Wheat DGAT1-2.pro		
	280																																																			
	280																																																			
	G	A	T	H	G	S	S	I	D	E	E	N	I	K	G	P	T	I	N	S	V	V	Y	F	M	L	A	P	T	L	C	Y	Q	P	S	Y	P	R	T	A	F	I	R	K	G	W	V	T	R	Q		Consensus #1
	300																																																			
	300																																																			
	G	A	T	H	G	S	S	I	D	E	E	N	I	K	G	P	T	I	N	S	V	V	Y	F	M	L	A	P	T	L	C	Y	Q	P	S	Y	P	R	T	A	F	I	R	K	G	W	V	T	R	Q		BB1295 USCNT SEQ-22.pro
	G	A	T	H	G	S	S	I	D	E	E	N	I	K	G	P	T	I	N	S	V	V	Y	F	M	L	A	P	T	L	C	Y	Q	P	S	Y	P	R	T	A	F	I	R	K	G	W	V	T	R	Q		PHF32068 Wheat DGAT1-2.pro
	330																																																			
	330																																																			
	L	I	K	C	V	F	T	G	L	M	G	F	I	E	Q	Y	I	N	P	I	V	Q	N	S	K	H	P	L	N	G	N	F	L	D	A	I	E	R	V	L	K	L	S	V	P	T	L	Y		Consensus #1		
	350																																																			
	350																																																			
301	L	I	K	C	V	F	T	G	L	M	G	F	I	E	Q	Y	I	N	P	I	V	Q	N	S	K	H	P	L	N	G	N	F	L	D	A	I	E	R	V	L	K	L	S	V	P	T	L	Y		BB1295 USCNT SEQ-22.pro		
301	L	I	K	C	V	F	T	G	L	M	G	F	I	E	Q	Y	I	N	P	I	V	Q	N	S	K	H	P	L	N	G	N	F	L	D	A	I	E	R	V	L	K	L	S	V	P	T	L	Y		PHF32068 Wheat DGAT1-2.pro		
	380																																																			
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	V	W	L	C	M	F	Y	S	F	F	H	L	W	L	N	I	L	A	E	L	R	F	G	D	R	E	F	Y	K	D	W	N	A	K	T	V	E	E	Y	W	R	M	N	M	P	V	H		Consensus #1			
	400																																																			
	400																																																			
351	V	W	L	C	M	F	Y	S	F	F	H	L	W	L	N	I																																				